

CHARACTERIZATION AND CELLULAR
LOCALIZATION OF MONOAMINES IN
THE HYPOTHALAMO-HYPOPHYSEAL
SYSTEM

BY

ANDERS BJÖRKLUND



Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41545309_0007

CHARACTERIZATION AND CELLULAR LOCALIZATION
OF MONOAMINES IN THE
HYPOTHALAMO-HYPOPHYSEAL SYSTEM

AKADEMISK AVHANDLING

som med vederbörligt tillstånd av Medicinska Fakulteten
i Lund för vinnande av medicine doktorsgrad kommer att
offentligen försvaras å Histologiska avdelningens föreläs-
ningssal (nya byggnaden), Institutionen för anatomi och histologi,
Lund, lördagen den 4 oktober 1969 kl. 9.15 (precis)

av

ANDERS BJÖRKLUND

Med. kand.,

Sm.

From the Institute of Anatomy and Histology
University of Lund, Lund, Sweden

CHARACTERIZATION AND CELLULAR LOCALIZATION
OF MONOAMINES IN THE
HYPOTHALAMO-HYPOPHYSEAL SYSTEM

BY

ANDERS BJÖRKLUND



Lund 1969

To my parents.

From the Institute of Anatomy and Histology
University of Lund, Lund, Sweden

CHARACTERIZATION AND CELLULAR LOCALIZATION
OF MONOMERES IN THE
HYPOTHALAMO-HYPOPHYSIAL SYSTEM

by

ANDERS FLORBERG



Lund 1959

The present summary is based on the following papers:

- I BJÖRKLUND, A., FALCK, B., and ROSENGREN, E., (1967) Monoamines in the pituitary gland of the pig. *Life Sci.* 6, 2103-2110.
- II BJÖRKLUND, A., and FALCK, B., (1968) An improvement of the histochemical fluorescence method for monoamines. Observations on varying extractability of fluorophores in different nerve fibres. *J. Histochem. Cytochem.* 16, 717-720.
- III BJÖRKLUND, A., (1968) Monoamine-containing fibres in the neurointermediate lobe of the pig and rat. *Z. Zellforsch.* 89, 573-589.
- IV BJÖRKLUND, A., ENEMAR, A., and FALCK, B., (1968) Monoamines in the hypothalamo-hypophyseal system of the mouse with special reference to the ontogenetic aspect. *Z. Zellforsch.* 89, 590-607.
- V BJÖRKLUND, A., EHINGER, B., and FALCK, B., (1968) A method for differentiating dopamine from noradrenaline in tissue sections by microspectrofluorometry. *J. Histochem. Cytochem.* 16, 262-270.
- VI BJÖRKLUND, A., FALCK, B., HROMEK, F., OWMAN, CH., and WEST, K.A., (1969) Identification and terminal distribution of the tubero-hypophyseal monoamine fibre systems in the rat by means of stereotaxic and microspectrofluorimetric techniques. *Brain Res.* In press.
- VII BJÖRKLUND, A., FALCK, B., and HÅKANSON, R., (1968) Histochemical demonstration of tryptamine. Properties of the formaldehyde-induced fluorophores of tryptamine and related indole compounds in models. *Acta physiol. scand. suppl.* 318.
- VIII BJÖRKLUND, A., and FALCK, B., (1969) Pituitary monoamines of the cat with special reference to the presence of an unidentified monoamine-like substance in the adenohypophysis. *Z. Zellforsch.* 93, 254-264.

- IX BJÖRKLUND, A., and FALCK, B., (1969) Histochemical characterization of a tryptamine-like substance stored in cells of the mammalian adenohypophysis. *Acta physiol, scand.* In press.

These papers will be referred to by their Roman numerals.

INTRODUCTION

The possible role of biogenic monoamines in the pituitary functions has been recognized in recent years. A most conspicuous finding was that reserpine, the well-known neuropharmacological agent which depletes the endogenous monoamine stores by blocking their intraneuronal storage sites, exerts multiple endocrine effects via the hypothalamo-hypophyseal system. In numerous investigations - excellently reviewed by Gaunt, Chart and Renzi (1961), Gaunt, Renzi and Chart (1962), and Gold and Ganong (1967) - this drug has been found to cause ACTH hypersecretion, lactation, pseudopregnancy, blockade of ovulation, and other endocrine changes. It seems reasonable to suppose that the alterations in the pituitary functions caused by reserpine are exerted through the depletion of monoamines from their storage sites. Experiments with monoamine oxidase (MAO) inhibitors have further supported the idea that monoamines play a role in the hypothalamo-hypophyseal functions. The MAO inhibitors which block the enzymatic oxidative breakdown of monoamines and cause an increase in the brain monoamine levels, also affect various pituitary functions (see Gold and Ganong 1967 and Ganong and Lorenzen 1967 for review of the literature). It is of special interest in this respect that MAO inhibitors prevent the major endocrine changes caused by reserpine administration, such as the increase in ACTH secretion (Gaunt et al. 1962, Martel et al. 1962, Mizuno et al. 1964), the inhibition of ovulation (Lippmann et al. 1967, Meyerson and Sawyer 1968), and pseudopregnancy (Lippmann et al. 1967). It is thus of great interest that specific monoamine fluorescence occurs in neurons and in endocrine cells of the hypothalamo-hypophyseal system, as demonstrated in studies with the Falck-Hillarp histofluorescence technique (Carlsson, Falck and Hillarp 1962, Fuxe 1963, 1964, 1965, Dahlström and Fuxe 1966). In the present studies, the cellular distribution and the identity of the various monoamines occurring in the hypothalamo-hypophyseal system have been studied in some mammalian species by means of histochemical techniques -

with special emphasis on microspectrofluorimetric analysis - in combination with biochemical determinations to provide a basis for a more detailed analysis of their functional significance in the neuroendocrine mechanisms of the hypothalamo-hypophyseal system (Paper I, III, IV, VI, VIII and IX).

In the histochemical fluorescence method of Falck and Hillarp (Falck 1962, Falck, Hillarp, Thieme and Torp 1962, Corrodi and Hillarp 1963, 1964) biogenic β -arylethylamines - such as dopamine, noradrenaline, adrenaline and 5-hydroxytryptamine - in freeze-dried tissues or enclosed in dried protein films react with gaseous formaldehyde to form intensely fluorescent products. This reaction involves an initial Pictet-Spengler condensation reaction followed by a dehydrogenation step, which is catalyzed by proteins or certain amino acids (Corrodi and Hillarp 1963, 1964, Jonsson 1967, Corrodi and Jonsson 1967). The possibilities of analysing the fluorophores, 6,7-dihydroxy-3,4-dihydroisoquinolines formed from the biogenic catecholamines and 6-hydroxy-3,4-dihydro- β -carboline formed from 5-hydroxytryptamine, by means of microspectrofluorimetry have been studied by van Orden, Vugman and Giarman (1965), by Ritzén and co-workers (Caspersson, Hillarp and Ritzén 1966), and by Jonsson (1967). They demonstrated that the fluorescent condensation products displayed excitation and emission spectra with well-defined and reproducible peak maxima, allowing the microspectrofluorimetric distinction between 5-hydroxytryptamine, on one hand, and the catecholamines on the other. However, the fluorophores of the various catecholamines - dopamine, noradrenaline and adrenaline - and dopa showed similar spectral characteristics.

The great demand for methods for the characterization and distinction of the several monoamine fluorophores on the cellular level initiated a study of the influence of variations in pH after the formaldehyde treatment on the spectral characteristics of the monoamine fluorophores and on the fluorescence yield (Paper V and VII). Hereby, an increase in the analytical value of the microspectrofluorimetric recordings was achieved, and thus it became possible to differentiate between the primary catechol-

amines dopamine and noradrenaline as well as between closely related indole compounds at the cellular level. Further, the possibilities of influencing the dehydrogenation step of the histochemical formaldehyde reaction was studied in order to increase the sensitivity of the method for certain tryptamine derivatives. Thus, a formaldehyde condensation procedure which is sensitive and probably very selective for the demonstration of tryptamine at the cellular level could be devised (Paper VII).

RESULTS

1. Occurrence of catecholamines and 5-hydroxytryptamine in the hypothalamo-hypophyseal system

Papers I, VI and VIII

Chemical fluorimetric determinations of dopamine, noradrenaline and adrenaline, and 5-hydroxytryptamine were performed according to Bertler et al. (1958) and Bertler (1961) on the pituitary gland of the pig (Paper I), cat (Paper VIII) and rat (Paper VI) and on the median eminence and hypothalamus of the rat (Paper VI). In the pituitary gland, separated from the median eminence and the infundibular stem, dopamine was found to be the predominating catecholamine in all three species. In the cat and rat, significant amounts of noradrenaline also occurred; in the cat, the noradrenaline concentration amounted to about 1/3 of the dopamine concentration, and in the rat, to about 1/2. In the pig pituitary, the regional distribution of the monoamines was studied (Paper I). Dopamine was almost exclusively localized in the neuro-intermediate lobe, where the average concentration was 0.41 g/g. The dopamine was most probably localized both in the neural lobe and in the pars intermedia. Low concentrations of 5-hydroxytryptamine (0.04-0.15 µg/g) and noradrenaline (0.01-0.05 µg/g) was found in all lobes of the pig pituitary, the amine thus having an even distribution throughout the several lobes. In the cat pituitary, on the other hand, notably high concentrations (M. V. 0.94 µg/g) of 5-hydroxytryptamine were recorded.

In the rat, the distribution of dopamine and noradrenaline was studied in three different regions of the hypothalamo-hypophyseal system (Paper VI): 1. Neuro-intermediate lobe. 2. Median eminence plus infundibular stem and arcuate nuclei. 3. The remainder of the hypothalamus. Both catecholamines occurred in significant amounts in all three preparations. The noradrenaline to dopamine ratio was appr. 1:2

in the neuro-intermediate lobe, appr. 1:1 in the median eminence preparation and appr. 3.5:1 in the remainder of the hypothalamus (cf. Table 1 in Paper VI). Thus, dopamine predominated in the neuro-intermediate lobe and noradrenaline in the remainder of the hypothalamus, whereas about equal amounts of the two catecholamines occurred in the median eminence preparation.

2. Demonstration of catecholamine-containing fibres in the pituitary gland and their morphological characteristics

Paper I, II, III, IV and VIII

The first histochemical investigation on the monoamines of the hypothalamo-hypophyseal system was performed by Carlsson, Falck and Hillarp (1962). They reported the occurrence of strong accumulation of fluorescence characteristic of catecholamines close to the portal vessels of the median eminence of the mouse; they also described fluorescent cell bodies in the vicinity of this region. These structures were later described in detail by Fuxe (1963, 1964) and Enemar and Falck (1964, 1965a) in various mammals. A rich adrenergic innervation of the pars intermedia was established by Fuxe (1964) and Dahlström and Fuxe (1966) but the neural lobe of a number of common laboratory mammals, including rat and cat, was reported to lack such innervation.

A special feature of the catecholamine-containing structures of the median eminence is that the amines or their formaldehyde-induced fluorophores have a strong tendency to diffuse, as reported by Carlsson et al. (1962), Fuxe (1964) and Enemar and Falck (1964, 1965a). Since this phenomenon could well be due to factors involved in the histotechnical procedure, an investigation into the extraction and diffusion phenomena of the formaldehyde-induced fluorophores in different nerve fibres during the various steps of the Falck-Hillarp method was undertaken (Paper II). It was found that the catecholamine fluorophores obtained in certain fibre structures - e. g. in the median eminence of the mouse (Paper IV, cf. above), in the neural lobe (Paper III, cf. below), and in the pancreas - were easily extracted from their tissue sites by xylene or hot paraffin, i. e. by two media which are used in the preparation of the specimens

for fluorescence microscopy. This contrasts sharply to the fluorophore obtained in peripheral adrenergic nerve fibres, which is fairly resistant to such media (cf. Falck and Torp 1961, Falck 1962, Corrodi et al. 1964). A conspicuous example of the varying extractability of the catecholamine fluorophores in different nerve fibres was found in the pituitary gland of the pig (Paper II and III): In the same sections, the fluorescence of the dopamine fibres in the pars intermedia showed a good resistance to hot paraffin and xylene, whereas the dopamine fluorophore in the fibres of the neural lobe was easily extracted.

Based on these experiments, a modified histochemical procedure was designed (Paper II), which reduces to a minimum the exposure of the specimens to hot paraffin and xylene. With this modification, a higher sensitivity and reproductiveness of the fluorescence method was achieved in many structures, e.g. in the neurohypophysis. Thus, a rich system of catecholamine-containing fibres could now be demonstrated in the neural lobe of the pig, rat (Paper I and III) and cat (Paper VIII); the presence of such fibres has been denied by earlier investigators. It is remarkable that only few catecholamine fibres were observed in the neural lobe of the mouse (Paper IV).

From a morphological point of view, three types of catecholamine fibres could be distinguished in the neural lobe: 1) A rich network of delicate varicose fibres distributed throughout the parenchyma of the lobe; these sometimes show direct continuity with the delicate catecholamine fibres surrounding the intermedia cells. 2) Catecholamine-containing droplet-like swellings or clusters of swellings; they probably constitute terminal swellings or end-apparatuses on smooth or varicose fibres. Such "droplet fibres" were also found among the cells of the pars intermedia and in the external and internal layers of the median eminence (Paper IV, cf. below). Such catecholamine-containing "droplet fibres" have not so far been observed outside the hypothalamo-hypophyseal system. 3) Coarse varicose fibres which were mainly localized around larger vessels, having the appearance of ordinary sympathetic vascular nerves. The experiments with sympathectomy revealed that at least some of them were of sympathetic origin.

The catecholamines in the median eminence of the mouse were found to be confined to fibre structures located in both the external and internal layers (Paper IV), in agreement with Fuxe (1964), Enemar and Falck (1965a) and Otake (1967). The zona externa showed the highest density of fibres; these had the appearance of delicate varicose fibres running perpendicularly to the surface and ending with a large fluorescent swelling in close contact with the capillaries of the portal vessels. The abundance and size of the terminal swellings varied notably from animal to animal. The catecholamine fibres were restricted to the brain tissue, and no sympathetic innervation of the portal vessels was observed. A rich adrenergic innervation occurred also in the internal layer, most prominently around and close to the so-called deep vessels in the subependymal part of the fibre layer of the caudal portion of the median eminence. In this area, "droplet fibres" similar to those in the neuro-intermediate lobe were regularly found.

Catecholamine-containing cell bodies occur in the n. arcuatus and in the n. periventricularis anterior (Paper IV, Fuxe 1964, Lichtensteiger and Langemann 1966, Otake 1967). These neurons probably contribute to the catecholamine innervation of the median eminence (Lichtensteiger and Langemann 1966, Fuxe and Hökfelt 1966). In addition, fluorescent cell bodies have sometimes been observed in n. premammillaris ventralis (Paper IV). It is remarkable that after administration of the catecholamine precursor DOPA in combination with an MAO-inhibitor, or after high doses of noradrenaline, catecholamine fluorescence occurred in a large number of neurons in this nucleus.

3. The ontogenetic appearance of the catecholamine-containing structures in the neurohypophysis and adjoining structures of the mouse

Paper IV

The first traces of monoamine fluorescence is seen in the brain anlage as early as the 12th day of the gestation period (the earliest stage included in the investigation). Fluorescent fibres of vari-

coarse appearance are seen in some hypothalamic nuclei in the 16-day embryo, and the adult pattern of the monoamine fibres in the hypothalamus is reached at birth. Thus, the hypothalamic monoamines appear prenatally, with two exceptions: the fluorescent cell bodies in the ventral hypothalamus, and the catecholamine fibres of the median eminence.

The first traces of specific, i.e., monoamine, fluorescence in the median eminence are seen in the embryos two or three days before birth, and a moderate number of coarse varicose fibres are seen at birth, mainly in the zona interna. A rich innervation of the internal layer is reached 3-5 days after birth. Characteristic delicate catecholamine fibres in the zona externa close to the primary portal capillaries are first seen towards the end of the first postnatal week. The number and the intensity of the fibres in the external layer increase during the second and third postnatal week, and about three weeks after birth, all features of the adult eminentia are seen. Catecholamine fluorescence in the cell bodies of the ventral hypothalamus could be traced back to the 17-day embryo. The number and intensity of these cell bodies approach the adult condition during the first postnatal week.

In the adult mouse, catecholamine fibres occurred in the pars intermedia and at the junction between pars intermedia and neural lobe, but only few such fibres could be detected in the neural lobe (Paper IV). Also specific fluorescence was detected in most intermedia cells (cf. below). The appearance of monoamines in the neuro-intermediate lobe is chiefly a postnatal process. The first fluorescent fibres and intermedia cells appear at birth, and about one week after birth, the neuro-intermediate lobe is similar to that of the adult animal.

To summarize: The catecholamines in the hypothalamic fibre structures were found to develop prenatally, whereas the monoamine structures in the deeper layers of the median eminence and in the neuro-intermediate lobe, as well as the fluorescent cell bodies of the ventral hypothalamus, cannot be demonstrated histo-

chemically until the first postnatal week, and the catecholamine structures in the zona externa not until the second and third postnatal weeks.

4. Microspectrofluorimetric characterization of dopamine and noradrenaline at the cellular level

Paper V

In their studies on the structure of the fluorophores formed in the histochemical reaction between primary catecholamines and formaldehyde, Corrodi and Jonsson (1965) found that in model systems rather vigorous treatment of the fluorophores with HCl or SOCl_2 converts the 4, 6, 7-trihydroxy-3, 4-dihydroisoquinoline fluorophore formed from noradrenaline to the fully aromatic 6, 7-dihydroisoquinoline. They also found that this reaction was not possible in case of the dopamine fluorophore, 6, 7-dihydroxy-3, 4-dihydroisoquinoline.

In our experiments (Paper V) we found that this conversion of the noradrenaline fluorophore can be brought about under considerably milder conditions. Thus, in the microspectrofluorimetric investigations with a Leitz microspectrograph suitably modified for analysis of monoamine fluorophores (Paper V), we found that microspectrofluorimetric differences can be readily achieved between the noradrenaline and the dopamine fluorophores under conditions that could be adapted to sections from freeze-dried tissues. In short, the fluorophores formed from noradrenaline and dopamine at neutral pH are the 4, 6, 7-trihydroxy-3, 4-dihydroisoquinoline and the 6, 7-dihydroxy-3, 4-dihydroisoquinoline, respectively, in their quinoidal form (Corrodi and Hillarp 1964, Jonsson 1966). These fluorophores, which are normally obtained in tissues, show indistinguishable spectral characteristics (Paper V, cf. Caspersson et al. 1966). Upon acidification with HCl-vapour, the dopamine fluorophore will be converted into the tautomeric non-quinoidal form, which exhibits an excitation maximum at $370 \text{ m}\mu$. The noradrenaline fluorophore will only transiently occur in its non-quinoidal form, but is rapidly converted upon prolonged treatment with HCl to the fully aromatic isoquinoline, having the excitation maximum at approxi-

Characteristics of the

and normal

In the first of these

in the first of these

formation of the

rather vigorous movement of the

area the 1,6,7-trihydroxy-

from norbornene to the full

also found that this reaction

1-norbornene, 6-

In the

the normal

number of

tions with

normal

metric

and

each

formation

by

isodup

William

obtained in

(type

the

quies

n

to

fully

mately 330 m μ . Thus, clearly different excitation spectra could be obtained from dopamine and noradrenaline upon the HCl-treatment, which allows the microspectrofluorimetric characterization of these amines at the cellular level. This method was also successfully applied to cell systems containing an identified catecholamine (Paper V): the mast cells in the bovine liver and the varicose nerve terminals in the pedal ganglion of the fresh-water bivalve (*Anodonta piscinalis*), which are known to contain dopamine, and the sympathetic nerve terminals in mammals, which are known to contain noradrenaline. The usefulness of this analytical histofluorescence technique for the distinction of the primary catecholamines has already been demonstrated in a number of studies on both nervous and non-nervous tissues (Paper VI, Cegrell 1968, Ehinger et al. 1968, Björklund et al. 1969a, b).

5. Identification and terminal distribution of tubero-hypophyseal dopamine and noradrenaline fibre systems

Paper VI

Few attempts have been made to elucidate the origin and course of different tubero-hypophyseal monoamine fibre systems. The lesion experiments performed by Fuxe and Hökfelt (1966) and the noradrenaline uptake studies performed by Lichtensteiger and Langemann (1966) suggested that the catecholamine-storing cell bodies found in the arcuate nuclei have their terminals in the pituitary, probably in the median eminence. Pharmacological experiments with α -methyl-meta-tyrosine and desimipramine (Carlsson et al. 1962, Fuxe, 1964, Fuxe et al. 1966) indicated the presence of dopamine in the median eminence.

In the present experiments (Paper VI) the monoamine-containing fibres to the median eminence and the neuro-intermediate lobe of the rat were studied with fluorescence microscopy, in combination with micro-knife lesions according to the technique introduced by Halasz and Pupp (1965). The origin and the course of the fibres were evaluated on the basis of monoamine accumulation proximal to the lesion and monoamine disappearance distal to the lesion. The nature of the monoamines stored in the various fibre systems was characterized with the microspectro-

fluorimetric technique that allows the distinction between dopamine and noradrenaline fibres.

Five groups of monoamine-containing axons were seen to enter the median eminence: a) A large group of dopamine axons which originate in cells of the arcuate nuclei and the ventral parts of the anterior periventricular nuclei; they enter the fibre layer of the median eminence close to the infundibular recess; b) A large group of noradrenaline axons which originate in areas outside the medio-basal hypothalamus; they enter the anterior median eminence from the lateral side close to the brain surface. In the internal layer and the deeper part of the external layer of the median eminence, they intermingle with the arcuato-hypophyseal dopamine fibres; c) Two minor groups of catecholamine axons: one reaches the median eminence from the anterior side, with an antero-posterior direction probably mingled with the supraopticohypophyseal tract; the other runs into the arcuate nucleus with a dorso-ventral course along and close to the third ventricle. The fibres could not be traced further; d) A small group of scattered axons containing an unidentified fluorogenic substance whose formaldehyde-induced fluorophore differed microspectrofluorimetrically from the catecholamines and 5-hydroxytryptamine.

Judging from the disappearance of monoamines distal to the lesion and from the chemical and microspectrofluorimetric analyses of the catecholamines in the median eminence and the neuro-intermediate lobe, the following conclusions concerning the terminal distribution of the catecholamine fibre systems could be drawn: The arcuato-hypophyseal dopamine neurons give rise to only part of the terminals of the zona externa of the median eminence and to most of the terminals in the zona interna. The tubero-hypophyseal noradrenaline fibres also have their terminals in the zona externa, and they contribute to the catecholamine innervation of the neuro-intermediate lobe.

Our investigation thus demonstrated two prominent neuron systems in the hypothalamo-hypophyseal complex: one containing dopamine, one containing noradrenaline. Moreover, the results suggest

the involvement of a third neuron system, possibly containing an indole derivative.

6. A sensitive histofluorescence method for the demonstration of tryptamine and closely related compounds at the cellular level

Paper VII

In the histochemical method of Falck and Hillarp, tryptamine reacts with formaldehyde to fluorescent products, but the fluorescence yield is low. Thus, under standard conditions, the fluorescence yield of equimolar amounts of dopamine and noradrenaline is 4-7 times higher than that of tryptamine (Paper VII, Caspersson et al. 1966, Ritzén 1967, Jonsson 1967). The high fluorescence yields of catecholamines are due to the dehydrogenation of the initially formed formaldehyde condensation products occurring readily in the dry state (Corrodi and Hillarp 1963, 1964). According to Hess and Udenfriend (1959) tryptamine in solution is easily condensed with formaldehyde to the non-fluorescent tetrahydronorharman, but the subsequent dehydrogenation to intensely fluorescent molecules requires the presence of oxidants. Based on the idea that the low fluorescence yield in the conventional formaldehyde reaction was far from optimal owing to a low degree of dehydrogenation, the possibility of introducing a fluorescence-promoting oxidation step in the histochemical formaldehyde reaction was investigated.

a) The combined formaldehyde-ozone reaction. When a gaseous oxidant (ozone) was introduced into the formaldehyde reaction vessel, a dramatic increase in the fluorescence yield of tryptamine was achieved. Thus, up to a certain concentration of ozone, the fluorescence yield of tryptamine progressively increased to reach about 13 times the yield obtained in normal air. This increase was obtained also when the ozone treatment was performed after the formaldehyde treatment, but it did not occur when the ozone treatment preceded the formaldehyde treatment. Thus, the effect of the ozone treatment may well be due to an increased oxidative dehydrogenation of the initially formed formaldehyde condensation product (tetrahydronorharman from tryptamine)

THE UNIVERSITY OF CHICAGO LIBRARY
1000 S. MICHIGAN AVE. CHICAGO, ILL. 60607
TEL. 778-4131

THE UNIVERSITY OF CHICAGO LIBRARY
1000 S. MICHIGAN AVE. CHICAGO, ILL. 60607
TEL. 778-4131

THE UNIVERSITY OF CHICAGO LIBRARY
1000 S. MICHIGAN AVE. CHICAGO, ILL. 60607
TEL. 778-4131

resulting in an increased yield of intensely fluorescent molecules.

In the combined formaldehyde-ozone reaction, none of the other tryptamine and phenylethylamine derivatives tested (tryptophan, N-methyltryptamine, indole-3-acetic acid, 5-hydroxytryptamine, 5-hydroxytryptophan, 5-methoxytryptamine, 5-methoxytryptophan, N,N-dimethyltryptamine, melatonin, adrenaline, noradrenaline, dopamine, dopa, and metaraminol) showed such an increase in the fluorescence yield. In fact, the fluorescence yields of the biogenic catecholamines and 5-hydroxytryptamine were reduced by about 80% compared with the yields in the conventional formaldehyde treatment. This decrease was presumably caused by an oxidative breakdown of the amines or of their fluorophores.

The combined formaldehyde and ozone treatments not only make possible a sensitive and probably very selective demonstration of tryptamine at the cellular level, but also have proved to be a valuable tool for the histochemical characterization of and differentiation between closely related indoleamines (Paper VII).

b) The influence of pH on the fluorescence yield. Only preliminary results from this investigation, which is still in progress, have so far been published (Paper VII).

In the model experiments, the tested tryptamine derivatives showed in general a higher fluorescence yield after conventional formaldehyde treatment when the buffer of the model solution had an acid pH. Tryptamine showed the most pronounced increase in the fluorescence yield - c. 18-fold at pH 1 compared with pH 7 - but a marked increase was also recorded for tryptophan and 5-hydroxytryptamine. In case of tryptamine and tryptophan, similar results were obtained when the models were acidified after the formaldehyde treatment by means of HCl-vapours or glacial acetic acid. Later experiments (Björklund, Falck and Stenevi to be published) showed that the presence of minute amounts of HCl in the reaction vessel catalyzes the histochemical formaldehyde reaction for many tryptamine and phenylethylamine derivatives and makes possible their demonstration at the cellular level with high sensitivity.

[illegible]

c) Tissue model experiments. The combined formaldehyde-ozone procedure and the acidification after conventional formaldehyde treatment have been successfully applied to tissues incubated in a tryptamine solution. It was also found that tryptamine concentrations too low to be detected with the Falck-Hillarp technique can readily be detected by these procedures.

7. Microspectrofluorimetric differentiation between tryptamine and other biogenic arylethylamines

Paper V and VII

Tryptamine and tryptophan can easily be distinguished from the biogenic catecholamines and 5-hydroxytryptamine, since differences are observed both in spectra of the fluorophores at neutral pH and in the spectral variations upon acidification and alkalization (Paper VII). Moreover, in contrast to tryptamine and tryptophan, the fluorescence yield of catecholamines and 5-hydroxytryptamine is considerably lower in the combined formaldehyde-ozone reaction than after formaldehyde treatment alone.

Tryptophan often yields a clearly yellow fluorophore which exhibits emission characteristics similar to the tryptamine fluorophore, but in this case marked differences can be obtained in the excitation spectra upon variation in pH. Furthermore, under certain conditions (formaldehyde treatment in air, low or moderate tryptophan concentration, dryer paraformaldehyde - see Paper VII) a blue fluorophore is obtained from tryptophan, which was never obtained from tryptamine. This offers possibilities of distinguishing tryptamine from tryptophan microspectrofluorimetrically. N-methyltryptamine was the only compound tested that showed spectral characteristics indistinguishable from those of tryptamine. However, the fluorescence yield of tryptamine was clearly higher than that of N-methyltryptamine upon combined formaldehyde-ozone treatment.

In conclusion, the above-mentioned procedures in combination with microspectrofluorimetric analyses provide sensitive means for the detection and characterization of tryptamine at the cellular level.

8. Demonstration and characterization of a tryptamine-like substance in cells of the mammalian pars distalis and pars intermedia

Paper VIII and IX

The occurrence of specific, i. e. formaldehyde-induced, fluorescence in cells of the mammalian adenohypophysis has been observed earlier (Paper I and IV, Pearse and McGregor 1964, Dahlström and Fuxe 1966). The nature of this fluorogenic substance remained obscure; the presence of 5-hydroxytryptamine (Pearse and McGregor 1964) as well as a primary catecholamine (Dahlström and Fuxe 1966) has thus been suggested. However, in the investigation on the regional distribution of catecholamines and 5-hydroxytryptamine in the pig pituitary (Paper I), only very low concentrations of these amines were found in the pars distalis, where many cells contain this fluorogenic substance.

In a combined chemical and fluorescence microscopical study on normal and reserpine-treated cats (Paper VIII) it was possible to show that no - or only little - catecholamine or 5-hydroxytryptamine but some other monoamine-like substance is stored in the fluorescent cells of the pars intermedia and pars distalis. Thus, upon reserpine treatment the pituitary catecholamines and 5-hydroxytryptamine are largely depleted, whereas the intensity (and spectral properties) of the cellular fluorescence remained unaffected by this treatment.

When the microspectrofluorimetric techniques and the formaldehyde-ozone and the acidification procedures were applied to the pituitary gland of the rat, cat, and pig (Paper VIII and IX), it was found that the characteristics of the fluorogenic substance stored in the adenohypophysis differed entirely from those of the biogenic catecholamines and 5-hydroxytryptamine. In fact, the substance could be transformed to a highly fluorescent derivative under the special reaction conditions required to obtain a high fluorescence yield of tryptamine, and showed microspectrofluorimetric characteristics indistinguishable from those of the tryptamine fluorophore according to all criteria available at present (Paper VII and IX). In Paper IX, the reliability of these histochemical criteria are discussed, and it is concluded that tryptamine or a closely related β -(3-indolyl)-ethylamine is stored in the fluorescent cells of the mammalian adenohypophysis.

GENERAL DISCUSSION

a) Median eminence. It is now generally accepted that the hypothalamus regulates the pituitary hormone secretion in mammals (cf. McCann et al. 1968, Szentágothai et al. 1968). This regulation appears to be mediated via the release of polypeptide substances from the median eminence into the portal vessels. Via the portal blood, these hypothalamic substances are transported to the pars distalis where they are supposed to stimulate or inhibit the secretion of various hormones (cf. Green and Harris 1947, McCann et al. 1968). The following will only treat the possible role of monoamines in these hypothalamo-hypophyseal regulation mechanisms.

There have appeared only few histophysiological studies on the arcuato-hypophyseal dopamine neuron system in which the Falck-Hillarp technique has been utilized. By means of quantitative microspectrophotometry, Lichtensteiger (1967, 1969) has made an elegant analysis of the variation in the catecholamine content of the fluorescent perikarya in the arcuate and anterior periventricular nuclei during the estrus cycle. This study revealed a rhythmic variation in the mean relative fluorescence intensity in these cell bodies, with the lowest intensities on diestrus day 1 and the highest intensities during estrus. However, the change in the frequency distribution of the cell intensities suggested that not all fluorescent cells followed this cyclic variation. A rhythmic variation during the estrus cycle was also found in the dopamine neurons of the substantia nigra, which are not directly associated with the hypothalamus, but this variation was different and considerably smaller. To judge from these results, it seems likely that the variation in the nerve cells of the arcuate and periventricular nuclei reflects a functional variation in these neurons during the estrus cycle. By means of mere visual observations, an increase in the fluorescence intensity of these perikarya has been reported also during pregnancy and pseudopregnancy (Fuxe, Hökfelt and Nilsson

1967) and after castration (Barry and Leonardelli 1968).

Apart from the arcuato-hypophyseal dopamine neuron system (Paper VI, Fuxe and Hökfelt 1966, Lichtensteiger and Langemann 1966) which most probably has its terminals in the external and internal layer of the median eminence (Paper VI), the existence of a tubero-hypophyseal neuron system was established, having its terminals in the external layer of the median eminence (Paper VI). The above-mentioned histofluorescence studies on the cell bodies of the arcuato-hypophyseal dopamine neurons apparently demonstrate a variation in the dopamine content of the region in relation to the reproductive functions. It is remarkable that such variations have been established also for the hypothalamic noradrenaline content. Thus, Stefano and Donoso (1967) found that the noradrenaline concentration varied during the estrus cycle in the anterior and middle hypothalamus (which included the median eminence region), but not in the posterior hypothalamus. The rhythm, however, was different from that found by Lichtensteiger (1967, 1969) for the dopamine neurons: the highest noradrenaline concentrations were found during proestrus, and the lowest during estrus. Stefano and Donoso point to the interesting parallelism between this variation in hypothalamic noradrenaline and that of hypothalamic LH-releasing factor (Chowess and McCann 1965) and that of pituitary and plasma concentrations of gonadotrophins (McCann and Ramirez 1964) during the estrus cycle. An increase in the noradrenaline concentration was found in the anterior hypothalamus (but not in the other parts) also after castration (Stefano et al. 1965, Donoso et al. 1967). On the basis of these various findings, it is tempting to suggest a hypothetical arrangement in which the tubero-hypophyseal noradrenaline neurons are somehow related to the pituitary secretion of FSH and/or LH, and the arcuato-hypophyseal dopamine neurons have a similar relation to the prolactin secretion.

Many investigations provide evidence of the involvement of the hypothalamic catecholamines in the pituitary secretion of gonadotrophic hormones. Fuxe, Hökfelt and Nilsson (1967) studied the catecholamine terminals in the median eminence by means of a tyrosine hydroxylase inhibitor in order to block the synthesis of the catecholamines. The

rate of disappearance of the catecholamine fluorescence after treatment with the inhibitor was used as a measure of the activity of the neurons, and they found that the fluorescence disappeared quicker during pregnancy and lactation and slower after castration than in the control rats. Coppola, Leonardi, Lippmann and co-workers (Coppola et al. 1965, 1966, Lippmann et al. 1967) showed that inhibitors of catecholamine synthesis (as well as reserpine, which has a less selective action on brain monoamines) induce pseudopregnancy and block ovulation in rats. Also local depletion of monoamines in the median eminence obtained with implantation of minute amounts of reserpine in this region, causes pseudopregnancy, as well as lactation and depletion of prolactin from the pituitary (Kanematsu and Sawyer 1963, van Maanen and Smelik 1968), which demonstrates that these effects are mediated via the hypothalamus (see also Kanematsu, Hilliard and Sawyer 1963). Thus, depletion of catecholamines from the tubero-hypophyseal systems appears to stimulate the prolactin secretion and suppress the secretion of FSH and/or LH. Of special interest and significance in this context are the experiments of Matsui (1967) with implantations of noradrenaline in the median eminence region. Although the material was too small to allow definite conclusions, the results suggested that the noradrenaline induced irregular estrus cycles, compared with the sham operated animals and with animals with noradrenaline implants outside the arcuate nucleus - median eminence region. Thus, the noradrenaline near the median eminence probably altered the gonadotrophin secretion from the pars distalis.

b) Neural lobe. The rich supply of dopamine-containing "droplet fibres" to the neural lobe (Paper I and III) is suggestive of a functional role of dopamine in the secretion of hormones from this lobe. However, no data are available on the effect of dopamine or related amines on the function of the neural lobe. Reserpine, which easily depletes dopamine from the neural lobe (Paper VIII), has clear effects on the water and electrolyte balance, but these effects are probably not mediated only via the pituitary (Gaunt et al. 1962). Chaudhury et al. (1962) showed that acute administration of reserpine causes a release of antidiuretic hormone (ADH) in the hydrated rat. Chronic administration of reserpine,

2011年12月15日

on the other hand, which produces a loss of function in monoamine neurons, has been reported to block the dehydration-induced ADH-release (Moses 1964), and to decrease the antidiuretic activity of the blood (Vial et al. 1957). In this context it should also be mentioned that adrenergic blocking agents blocked the release of ADH upon hypothalamic (Fang et al. 1962) or ulnar nerve stimulation (Mills and Wang 1964) in the dog, and reduced the increase in AD-activity in the blood on injection of hypertonic saline in the rat (de Wied and László 1967). Also the oxytocin response in the so-called milk let-down reflex was reduced by adrenergic blocking agents (Grosvenor and Turner 1957).

When considering the structural basis for the above-mentioned pharmacological effects, it should be remembered that not only the neural lobe but also the fibre layer of the zona externa of the median eminence and the supraoptic and paraventricular nuclei (Carlsson et al. 1962, Fuxe 1965) receive catecholamine-containing varicose fibres. Thus monoamine mechanisms may operate at several different levels of the hypothalamo-hypophyseal neurosecretory neurons. The control of the neurosecretory neurons could thus be arranged in a manner similar to that proposed by Knowles (1965) for the MSH-producing intermedia cells of the dogfish: one group of nerves at the level of the perikaryon regulating the polypeptide synthesis, and one group of nerves at the level of the neural lobe regulating the polypeptide release. Since the catecholamine fibres in the supraoptic and paraventricular nuclei probably store noradrenaline (Fuxe 1965) and the fibres in the neural lobe store dopamine (Paper I, III and VI) the control of polypeptide synthesis and release could also be attributed to noradrenaline and dopamine, respectively.

c) Adenohypophysis. Also the pars intermedia receive catecholamine-containing fibres, most probably nerve terminals (Paper I, III, IV, VI and VIII). They show morphological features similar to the fibres of the neural lobe, i.e. both fine varicose fibres and "droplet fibres" occur, and fibres passing between the two lobes are readily observed in the fluorescence microscope (Paper III). Both dopamine and noradrenaline fibres were found in the rat pars intermedia (Paper VI), whereas most, possibly all, fibres in the pig pars intermedia store dopamine (Paper I,

unpublished observations). The endocrine function of the mammalian pars intermedia as well as the possible role of its catecholamine innervation is obscure. However, a catecholamine innervation of central origin similar to that found in mammals has been demonstrated in amphibians (Enemar and Falck 1965b, Enemar, Falck and Iturriza 1967) and pharmacological (e.g. with reserpine) and physiological experiments have provided evidence that these nerves exert an inhibitory control of the MSH-production (Enemar and Falck 1965b, Iturriza 1966, 1967, 1969). According to Tomatis and Taleisnik (1968), reserpine depletes MSH from the pars intermedia of both toads and rats, and causes an increase in the MSH-release - inhibiting activity of the stalk-median eminence region in the rat. The authors suggested that reserpine increased the MSH-secretion by blocking the release of MSH-release-inhibiting factor which thereby accumulated in the hypothalamus. This suggests an interaction between the monoamines and the MSH-release-inhibiting factor in the control of the pars intermedia.

Monoamine stores have been found in many endocrine cell systems. Thus, in various mammals, dopamine and 5-hydroxytryptamine have been demonstrated in the endocrine cells of the islets of Langerhans (Falck and Hellman 1963, Cegrell 1968), and a storage of 5-hydroxytryptamine (as well as related indole compounds) has been found in the pinealocytes (Quay and Halevy 1962, Bertler et al. 1963, 1964, Quay 1965). 5-Hydroxytryptamine in high concentrations is also found in the enterochromaffin cells of the digestive tract (Pearse 1961, Vialli 1966) and in the parafollicular cells of the sheep thyroid gland (Falck et al. 1964). In the rat stomach, histamine-storing cells resembling the enterochromaffin cells have also been described (Thunberg 1967, Håkanson and Owman 1967, Håkanson et al. 1967). Thus, it is reasonable to suppose that the monoamines have a functional significance in many endocrine cell systems, above all perhaps those which secrete polypeptide hormones (cf. Pearse 1966, 1968, Falck and Owman 1968). From this point of view, it is of considerable interest that substances histochemically similar to the known biogenic amines have been detected in the adenohypophyseal cells (Paper VIII and IX).

The storage of a tryptamine-like substance in the intermediate cells is a new feature of these cells, which opens up possibilities for histophysiological studies of this relatively unknown endocrine cell system in mammals. The tryptamine-like substance was also found in cells of the pars distalis, which have been claimed to be identical with the ACTH-producing cells (Pearse and McGregor 1964). It is interesting to note that α -ethyltryptamine and related compounds (including tryptamine) have been tested for their ability to inhibit ACTH-secretion in surgically stressed dogs (Tullner and Hertz 1964, Ganong et al. 1965, Lorenzen et al. 1965). The inhibition produced by these substances, however, was found to be mediated via the effect on the blood pressure, and when this was kept constant by bleeding the animal, the inhibition was no longer recorded (Ganong et al. 1967).

Dopamine, noradrenaline, adrenaline, and 5-hydroxytryptamine, which can be visualized with the Falck-Hillarp method, are well known to occur in mammalian tissues. However, there is evidence that indolylethylamines not identical with 5-hydroxytryptamine occur in amphibians (Erspamer 1966), in evertebrates (Carlisle 1956, 1963, Kerkut and Price 1963, 1964), and in the mammalian pineal gland (Quay and Halevy 1962, Quay 1965) and there is evidence that phenylethylamines different from the biogenic catecholamines occur in mammalian nervous tissues (Häggendal 1963, Spector et al. 1963, Carlsson and Waldeck 1964, Molinoff and Axelrod 1969). In the present studies, three different fluorogenic substances, not identical with the biogenic catecholamines or 5-hydroxytryptamine, were found in the hypothalamo-hypophyseal system. In the microspectrofluorimetric analysis, one of these was found to be very similar to tryptamine. In our opinion, the high sensitivity and precision of the analytical histofluorescence techniques will make possible further exploration of hitherto unknown monoamine systems.

ACKNOWLEDGEMENTS

I wish to express my deep gratitude to Assistant Professor Bengt Falck, my teacher and co-worker, to Professor Anders Enemar, who introduced me to the vast field of neuroendocrinology, and to Docent Berndt Ehinger, who taught me the microspectrofluorimetric work. Without their enthusiasm and guidance, these studies would not have been completed. It has been a privilege for me to work and co-operate within the monoamine research group at the Institute of Anatomy and Histology, University of Lund.

The project has been supported by grants from the faculty of Medicine, University of Lund, from the Swedish Natural Research Council (No. 99-35), from the United States Public Health Service (No. 06701-03), from the Ford Foundation (No. 68-383), and was carried out within a research organization sponsored by the Swedish Medical Research Council (Projects No. B69-14X-56-05C and B69-14X-712-04C).

REFERENCES

- BARRY, J. et J. LEONARDELLI, Etude comparée des neurones et des fibres monoaminergiques de la région tubéro-infundibulaire chez le cobaye mâle normal ou castré. *C.R. Acad. Sci. Ser. D.* 1968. 266. 15-17.
- BERTLER, Å., Effect of reserpine on the storage of catecholamines in brain and other tissues. *Acta physiol. scand.* 1961. 51. 75-83.
- BERTLER, Å., A. CARLSSON, E. ROSENGREN and B. WÄLDECK, A method for the fluorimetric determination of adrenaline, noradrenaline and dopamine in tissues. *Kungl. Fysiogr. Sällsk. Lund Förh.* 1958. 28. 121-123.
- BERTLER, Å., B. FALCK and CH. OWMAN, Cellular localization of 5-hydroxytryptamine in the rat pineal gland. *Kungl. Fysiogr. Sällsk. Lund Förh.* 1963. 33. 13-16.
- BERTLER, Å., B. FALCK and CH. OWMAN, Studies on 5-hydroxytryptamine stores in pineal gland of rat. *Acta physiol. scand.* 1964. 63. suppl. 239.
- BJÖRKLUND, A., L. CEGRELL, B. FALCK, M. RITZÉN and E. ROSENGREN, Dopamine-containing cells in sympathetic ganglia. *Acta physiol. scand.* 1969a. In press.
- BJÖRKLUND, A., B. FALCK and N. KLEMM, Microspectrofluorimetric and chemical investigation of catecholamine-containing structures in the thoracic ganglia of trichoptera (Insecta). 1969b. To be published.
- CARLISLE, D.B., An indole-alkylamine regulating heart-beat in crustacea. *Biochem. J.* 1956. 63. 32-33.
- CARLISLE, D.B., The neurohumoral control of heart rate in crustaceans. In, *Comparative neurochemistry*. Ed.: D. Richter. Pergamon Press, Oxford. 1964. 323-328.
- CARLSSON, A., B. FALCK and N.-Å. HILLARP, Cellular localization of brain monoamines. *Acta physiol. scand.* 1962. 56. suppl. 196.
- CARLSSON, A. and B. WÄLDECK, A method for the fluorimetric determination of 3-methoxytyramine in tissues and the occurrence of this amine in brain. *Scand. J. Clin. Lab. Invest.* 1964. 16. 133-138.
- CASPERSSON, T., N.-Å. HILLARP and M. RITZÉN, Fluorescence microspectrophotometry of cellular catecholamines and 5-hydroxytryptamine. *Exp. Cell Res.* 1966. 42. 415-428.
- CEGRELL, L., The occurrence of biogenic monoamines in the mammalian endocrine pancreas. *Acta physiol. scand.* 1968. suppl. 314.
- CHAUDHURY, R.R., M. ROY CHAUDHURY and F.C. LU, The mechanism of the reserpine-induced antidiuresis in the rat. *Can. J. Biochem. Physiol.* 1962. 40. 1465-1472.
- CHOWERS, I. and S.M. McCANN, Content of luteinizing hormone-releasing factor and luteinizing hormone during the estrous cycle and after changes in gonadal steroid titers. *Endocrinology* 1965. 76. 700-708.

- COPPOLA, J. A., R. G. LEONARDI and W. LIPPMANN, Ovulatory failure in rats after treatment with brain norepinephrine depletors. *Endocrinology* 1966. 78. 225-228.
- COPPOLA, J. A., R. G. LEONARDI, W. LIPPMANN, J. W. PERRINE and I. RINGLER, Induction of pseudopregnancy in rats by depletors of endogenous catecholamines. *Endocrinology* 1965. 77. 485-490.
- CORRODI, H. und N. -Å. HILLARP, Fluoreszenzmethoden zur histochemischen Sichtbarmachung von Monoaminen. 1. Identifizierung der fluoreszierenden Produkte aus Modellversuchen mit 6, 7-Dimethoxyisochinolinderivaten und Formaldehyd. *Helv. Chim. Acta* 1963. 46. 2425-2430.
- CORRODI, H. und N. -Å. HILLARP, Fluoreszenzmethoden zur histochemischen Sichtbarmachung von Monoaminen. 2. Identifizierung des fluoreszierenden Produktes aus Dopamin und Formaldehyd. *Helv. Chim. Acta* 1964. 47. 911-918.
- CORRODI, H., N. -Å. HILLARP and G. JONSSON, Fluorescence methods for the histochemical demonstration of monoamines. 3. Sodium borohydride reduction of the fluorescent compounds as a specificity test. *J. Histochem. Cytochem.* 1964. 12. 582-586.
- CORRODI, H. and G. JONSSON, Fluorescence methods for the histochemical demonstration of monoamines. 4. Histochemical differentiation between dopamine and noradrenaline in models. *J. Histochem. Cytochem.* 1965. 13. 484-487.
- CORRODI, H. and G. JONSSON, The formaldehyde fluorescence method for the histochemical demonstration of biogenic monoamines. A review on the methodology. *J. Histochem. Cytochem.* 1967. 15. 65-78.
- DAHLSTRÖM, A. and K. FUXE, Monoamines and the pituitary gland. *Acta Endocrinol.* 1966. 51. 301-314.
- DE WIED, D. and F. A. LASZLO, Effect of autonomic blocking agents on ADH-release induced by hyperosmoticity. *J. Endocrinol.* 1967. 37. XVI.
- DONOSO, A. O., F. J. E. STEFANO, A. M. BISCARDI and J. CUKIER, Effects of castration on hypothalamic catecholamines. *Amer. J. Physiol.* 1967. 212. 737-739.
- EHINGER, B., B. FALCK, H. PERSSON and B. SPORRONG, Adrenergic and cholinesterase-containing neurons of the heart. *Histochemie* 1968. 16. 197-205.
- ENEMAR, A. and B. FALCK, Cellular localization of brain monoamines. *Progr. in Brain Res.* 1964. 8. 28-44.
- ENEMAR, A. and B. FALCK, In: Owman, Ch. and Falck, B.: Localization of neuronal monoamines at the cellular level. Paper presented at Proc. Second Int. Pharmacol. Meeting Prague 1963. Pergamon Press, Oxford 1965a. 3. 165-183.
- ENEMAR, A. and B. FALCK, On the presence of adrenergic nerves in the pars intermedia of the frog, *Rana temporaria*. *Gen. Comp. Endocrinol.* 1965b. 5. 577-583.
- ENEMAR, A., B. FALCK and F. C. ITURRIZA, Adrenergic nerves in the pars intermedia of the pituitary in the toad, *Bufo Arenarum*. *Z. Zellforsch.* 1967. 77. 325-329.
- ERSPAMER, V., Occurrence of indolealkylamines in nature. In: *Handbuch der experimentellen Pharmakologie*. Springer. Berlin-Heidelberg-New York. 1966. 19. 132-181.

- FALCK, B., Observations on the possibilities of the cellular localization of monoamines by a fluorescence method. *Acta physiol. scand.* 1962. 56. suppl. 197.
- FALCK, B., N.-Å. HILLARP, G. THIEME and A. TORP, Fluorescence of catechol amines and related compounds condensed with formaldehyde. *J. Histochem. Cytochem.* 1962. 10. 348-354.
- FALCK, B., B. LARSON, C.v. MECKLENBURG, E. ROSENGREN and K. SVENAEUS, On the presence of a second specific cell system in mammalian thyroid gland. *Acta physiol. scand.* 1964. 62. 491-492.
- FALCK, B. and CH. OWMAN, 5-Hydroxytryptamine and related amines in endocrine cell systems. *Adv. Pharmacol.* 1968. 6. 211-231.
- FALCK, B. and A. TORP, New evidence for the localization of nor-adrenaline in the adrenal medulla. *Med. Exp.* 1961. 5. 429-432.
- FANG, H.S., H.M. LIU and S.C. WANG, Liberation of antidiuretic hormone following hypothalamic stimulation in the dog. *Amer. J. Physiol.* 1962. 202. 212-216.
- FUXE, K., Cellular localization of monoamines in the median eminence and in the infundibular stem of some mammals. *Acta physiol. scand.* 1963. 58. 383-384.
- FUXE, K., Cellular localization of monoamines in the median eminence and the infundibular stem of some mammals. *Z. Zellforsch.* 1964. 61. 710-724.
- FUXE, K., Evidence for the existence of monoamine neurons in the central nervous system. IV. Distribution of monoamine nerve terminals in the central nervous system. *Acta physiol. scand.* 1965. 64. suppl. 247.
- FUXE, K., B. HAMBERGER and T. MALMFORS, Inhibition of amine uptake in tubero-infundibular dopamine neurones and in catecholamine cell bodies of the area postrema. *J. Pharm. Pharmacol.* 1966. 18. 543-544.
- FUXE, K. and T. HÖKFELT, Further evidence for the existence of tubero-infundibular dopamine neurons. *Acta physiol. scand.* 1966. 66. 245-246.
- FUXE, K., T. HÖKFELT and O. NILSSON, Activity changes in the tubero-infundibular dopamine neurons of the rat during various states of the reproductive cycle. *Life Sci.* 1967. 6. 2057-2061.
- GANONG, W.F. and L. LORENTZEN, Brain neurohumors and endocrine function. In, *Neuroendocrinology*. Eds.: L. Martini and W.F. Ganong. Academic Press. New York and London. 1967. 2. 583-640.
- GANONG, W.F., A.T. BORYCZKA, L.C. LORENZEN and A.S. EGGE, Lack of effect of α -ethyltryptamine on ACTH secretion when blood pressure is held constant. *Proc. Soc. Exp. Biol. Med.* 1967. 124. 558-559.
- GANONG, W.F., B.L. WISE, R. SHACKLEFORD, A.T. BORYCZKA and B. ZIPF, Site at which α -ethyltryptamine acts to inhibit the secretion of ACTH. *Endocrinology* 1965. 76. 526-530.
- GAUNT, R., J.J. CHART and A.A. RENZI, Endocrine pharmacology. This undeveloped field provides broad possibilities in experimental and applied therapeutics. *Science*. 1961. 133. 613-621.

- GAUNT, R., A.A. RENZI and J.J. CHARO, Endocrine pharmacology of methyl reserpate derivatives. *Endocrinology* 1962. 71. 527-535.
- GOLD, E.M. and W.F. GANONG, Effects of drugs on neuroendocrine processes. In, *Neuroendocrinology*. Eds.: L. Martini and W.F. Ganong. Academic Press. New York and London. 1967. 2. 377-437.
- GREEN, J.D. and G.W. HARRIS, The neurovascular link between the neurohypophysis and adenohypophysis. *J. Endocrinol.* 1947. 5. 136-146.
- GROSVENOR, C.E. and C.W. TURNER, Evidence for adrenergic and cholinergic components in milk let-down reflex in lactating rat. *Proc. Soc. Exp. Biol. Med.* 1957. 95. 719-722.
- HALASZ, B., and L. PUPP, Hormone secretion of the anterior pituitary gland after physical interruption of all nervous pathways to the hypophysiotrophic area. *Endocrinology* 1965. 77. 553-562.
- HESS, S.M. and S. UDENFRIEND, A fluorimetric procedure for the measurement of tryptamine in tissues. *J. Pharmacol. exp. Ther.* 1959. 127. 175-177.
- HÅKANSON, R. and CH. OWMAN, Concomitant histochemical demonstration of histamine and catecholamines in enterochromaffin-like cells of gastric mucosa. *Life Sci.* 1967. 6. 759-766.
- HÅKANSON, R., CH. OWMAN and N.-O. SJÖBERG, Cellular stores of gastric histamine in the developing rat. *Life Sci.* 1967. 6. 2535-2543.
- HÄGGENDAL, J., The presence of 3-O-methylated noradrenaline (normetanephrine) in normal brain tissue. *Acta physiol. scand.* 1963. 59. 370-375.
- ITURRIZA, F.C., Monoamines and control of the pars intermedia of the toad pituitary. *Gen. Comp. Endocrinol.* 1966. 6. 19-25.
- ITURRIZA, F.C., The secretion of intermedin in autotransplants of pars intermedia growing in the anterior chamber of intact and sympathectomized eyes of the toad. *Neuroendocrinology* 1967. 2. 11-18.
- ITURRIZA, F.C., Further evidences for the blocking effect of catecholamines on the secretion of melanocyte-stimulating hormone in toads. *Gen. Comp. Endocrinol.* 1969. 12. 417-426.
- JONSSON, G., Fluorescence studies on some 6, 7-substituted 3, 4-dihydroisoquinolines formed from 3-hydroxytyramine (dopamine) and formaldehyde. *Acta chem. scand.* 1966. 20. 2755-2762.
- JONSSON, G., Fluorescence methods for the histochemical demonstration of monoamines. VII. Fluorescence studies on biogenic monoamines and related compounds condensed with formaldehyde. *Histochemie* 1967. 8. 288-296.
- JONSSON, G., The formaldehyde fluorescence method for the histochemical demonstration of biogenic monoamines. M.D. thesis, Stockholm 1967.
- KANEMATSU, S., J. HILLIARD and C.H. SAWYER, Effect of reserpine on pituitary prolactin content and its hypothalamic site of action in the rabbit. *Acta Endocrinol.* 1963. 44. 467-474.
- KANEMATSU, S. and C.H. SAWYER, Effects of intrahypothalamic implants of reserpine on lactation and pituitary prolactin content in the rabbit. *Proc. Soc. Exp. Biol. Med.* 1963. 113. 967-969.
- KERKUT, G.A. and M.A. PRICE, 6 HT in crab heart. *Life Sci.* 1963. no. 2. 129-130.

- KERKUT, G.A. and M.A. PRICE, Chromatographic separation of cardiaccelerators (6HT and a mucopeptide) from Carcinus Heart. *Comp. Biochem. Physiol.* 1964. 11. 45-52.
- KNOWLES, F., Evidence for a dual control by neurosecretion of hormone synthesis and hormone release in the pituitary of the dogfish *Scylliorhinus Stellaris*. *Phil. Trans. Roy. Soc. London B.* 1965. 249. 435-456.
- LICHTENSTEIGER, W., Mikrofluorimetrische Studien an katecholaminhaltigen hypothalamischen Nervenzellen der Ratte in den verschiedenen Phasen des viertägigen Oestruszyklus. *Helv. Physiol. Acta* 1967. 25. CR423-CR425.
- LICHTENSTEIGER, W., Cyclic variations of catecholamine content in hypothalamic nerve cells during the estrous cycle of the rat, with a concomitant study of the substantia nigra. *J. Pharmacol. exp. Ther.* 1969. 165. 204-215.
- LICHTENSTEIGER, W. and H. LANGEMANN, Uptake of exogenous catecholamines by monoamine-containing neurons of the central nervous system: uptake of catecholamines by arcuato-infundibular neurons. *J. Pharmacol. exp. Ther.* 1966. 151. 400-408.
- LIPPMANN, W., R. LEONARDI, J. BALL and J.A. COPPOLA, Relationship between hypothalamic catecholamines and gonadotrophin secretion in rats. *J. Pharmacol. exp. Ther.* 1967. 156. 258-266.
- LORENZEN, L.C., B.L. WISE and W.F. GANONG, ACTH-inhibiting activity of drugs related to α -ethyltryptamine: relation to pressor activity. *Fed. Proc.* 1965. 24. 128.
- MARTEL, R.R., E.O. WESTERMANN and R.P. MAICKEL, Dissociation of reserpine-induced sedation and ACTH hypersecretion. *Life Sci.* 1962. no. 4. 151-155.
- MATSUI, T., Effects on the rat estrous cycle of implants of norepinephrine placed in the median eminence. *Ann. Zool. Japon.* 1967. 40. 74-81.
- McCANN, S.M., A.P.S. DHARIWAL and J.C. PORTER, Regulation of the adenohypophysis. *Ann. Rev. Physiol.* 1968. 30. 589-640.
- McCANN, S.M. and V.D. RAMIREZ, The neuroendocrine regulation of hypophyseal luteinizing hormone secretion. *Rec. Progr. Hormone Res.* 1964. 20. 131-170.
- MEYERSON, B.J. and C.H. SAWYER, Monoamines and ovulation in the rat. *Endocrinology* 1968. 83. 170-176.
- MILLS, E. and S.C. WANG, Liberation of antidiuretic hormone: Pharmacologic blockade of ascending pathways. *Amer. J. Physiol.* 1964. 207. 1405-1410.
- MIZUNO, H., P.K. TALWALKER and J. MEITES, Inhibition of mammary secretion in rats by iproniazid. *Proc. Soc. Exp. Biol. Med.* 1964. 115. 604-607.
- MOLINOFF, P. and J. AXELROD, Octopamine: Normal occurrence in sympathetic nerves of rats. *Science* 1969. 164. 428-429.
- MOSES, A.M., Inhibition of vasopressin release in rats by chlorpromazine and reserpine. *Endocrinology* 1964. 74. 889-893.
- ODAKE, G., Fluorescence microscopy of the catecholamine-containing neurons of the hypothalamohypophyseal system. *Z. Zellforsch.* 1967. 82. 46-64.

- PEARSE, A.G.E., *Histochemistry, theoretical and applied*. Churchill. London 1961. 641-652.
- PEARSE, A.G.E., Common cytochemical and ultrastructural characteristics of cells producing polypeptide hormones (the APUD series) and their relevance to thyroid and ultimobranchial C cells and calcitonin. *Proc. Roy. Soc. B.* 1968. 170. 71-80.
- PEARSE, A.G.E., 5-Hydroxytryptophan uptake by dog thyroid C cells and its possible significance in polypeptide hormone production. *Nature* 1966. 211. 598-600.
- PEARSE, A.G.E. and M.M. MCGREGOR, Functional cytology of the pituitary gland. *Ann. Rep. Brit. Emp. Cancer Camp.* 1964. 2. 665-666.
- QUAY, W.B., Indole derivatives of pineal and related neural and retinal tissues. *Pharmacol. Rev.* 1965. 17. 321-345.
- QUAY, W.B. and A. HALEVY, Experimental modification of the rat pineal's content of serotonin and related indole amines. *Physiol. Zoöl.* 1962. 35. 1-7.
- RITZÉN, M., Quantitative fluorescence microspectrophotometry of 5-hydroxytryptamine-formaldehyde products in models and in mast cells. *Exp. Cell. Res.* 1967. 45. 178-194.
- SPECTOR, S., K. MELMON, W. LOVENBERG and A. SJOERDSMA, The presence and distribution of tyramin in mammalian tissues. *J. Pharmacol. exp. Ther.* 1963. 140. 229-235.
- STEFANO, F.J.E. and A.O. DONOSO, Norepinephrine levels in the rat hypothalamus during the estrous cycle. *Endocrinology* 1967. 81. 1405-1406.
- STEFANO, F.J.E., A.O. DONOSO and J. CUKIER, Hypothalamic nor-adrenaline changes in ovariectomized rats. *Acta physiol. Latino Amer.* 1965. 15. 425-427.
- SZENTÁGÓTHAI, J., B. FLERKÓ, B. MESS and B. HALÁSZ, Hypothalamic control of the anterior pituitary. *Akadémiai Kiadó. Budapest* 1968.
- TOMATIS, M.E. and S. TALEISNIK, Influence of reserpine on the pituitary content of melanocyte-stimulating hormone and on hypothalamic factors which affect its release. *J. Endocrinol.* 1968. 42. 505-512.
- TULLNER, W.W. and R. HERTZ, Suppression of corticosteroid production in the dog by monase. *Proc. Soc. Exp. Biol. Med.* 1964. 116. 837-840.
- THUNBERG, R., Localization of cells containing and forming histamine in the gastric mucosa of the rat. *Exp. Cell. Res.* 1967. 47. 108-115.
- VAN MAANEN, J.H. and P.G. SMELIK, Induction of pseudopregnancy in rats following local depletion of monoamines in the median eminence of the hypothalamus. *Neuroendocrinology* 1968. 3. 177-186.
- VAN ORDEN, L.S., I. VUGMAN and N.J. GIARMAN, 5-Hydroxytryptamine in single neoplastic mast cells: A microscopic spectrofluorometric study. *Science* 1965. 148. 642-644.
- VIAL, S.V., H. CROXATTO and L. BARNAFI, Influence of reserpine and apresoline on renal response to renin and on antidiuretic potency of the blood of normal rats. *J. Appl. Physiol.* 1957. 11. 227-230.

VIALLI, M., Histology of the enterochromaffin cell system. In, Handbuch der experimentellen Pharmakologie. Springer. Berlin-Heidelberg-New York. 1966. 19. 1-65.

